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Methods for Distinguishing Sources of Nutmeg or Mace

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Nutmeg and mace are important spices used for flavoring many food products. In addition to the ground spices themselves, the volatile oil obtained by steam distillation is used commercially in many processed foods, especially baked goods and other desserts, and in various blended sauces.

Both nutmeg and mace come from the fruit of the same tree, *Myristica fragrans;* nutmeg is the seed and mace the covering (arillode) enveloping the nutmeg. Since they are so closely associated in nature, it is not

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surprising that the volatile oils derived from nutmeg and mace are very similar in odor and flavor. Indeed, they can be used interchangeably to some extent, although price factors dictate greater use of nutmeg.

Nutmeg and mace are imported from two main growing areas: The East Indies, where nutmegs were first cultivated, and the West Indies, especially the island of Granada, where since World War II more and more of these spices have been grown and processed. In the trade, East Indian nutmeg and mace are considered to be of higher quality than West Indian varieties. Yet half of the nutmeg imported into the United States comes from the West Indies, largely because of the higher cost of East Indian spice. Because of these price and quality differences, it is important to have a reliable method for distinguishing between spices and oils from the two areas.

West Indian and East Indian oils differ somewhat in their physical properties, such as density, refractive index, optical rotation, etc. (4), but since the ranges of these values are wide and overlap, it is frequently impossible to detect the source of a sample on the basis of physical properties alone.

As a consequence of the possibility of misrepresentation, as well as a desire for more specific methods of analysis for spices, a project was initiated to develop methods for distinguishing between East Indian and West Indian nutmegs. The project was part of a collaborative analytical research program undertaken by the Department of Agriculture and the American Spice Trade Association. Previous studies of the chemical composition of nutmeg oils (4, 6) have shown the presence of large amounts of terpenes and many minor constituents, some present only in traces. Power and Salway (6) found traces of several phenols in nutmeg oil, among which they identified eugenol and isoeugenol. Paper partition chromatography (2, 3) of basic extracts of nutmeg oils confirmed the presence of phenols, and study of the patterns obtained by chromatography of the dyes derived from coupling the phenols with a diazonium salt (2) revealed marked qualitative and quantitative differences depending on the origin of the oil.

The methods described below are based on these differences in phenol content and make possible a positive identification of the source of a particular batch of nutmeg or nutmeg oil. The methods are equally applicable to mace or mace oil.

METHOD

Apparatus and Reagents

a. *Centrifuge.*—Tabletop model with 12 ml conical centrifuge tubes.

b. *Spectophotometer.*—Beckman Model DU[1] or other type capable of measuring absorbance at 398 mμ; 1 cm cells.

c. *Filter Paper.*—Whatman No. 1, 1×13 cm strips.

d. *p-Nitroaniline.*—Solid, recrystallized.

e. *Sulfuric Acid Solutions.*—6*N* (concentrated H_2SO_4 diluted 1+5); and 24*N* (concentrated H_2SO_4 diluted 2+1).

f. *Sodium Hydroxide.*—Solid pellets, reagent grade; and 10% aqueous solution.

g. *Sodium Nitrite Solution.*—0.2*M* solution, freshly prepared.

h. *Sodium Sulfate.*—Solid, anhydrous.

i. *Chloroform.*—ACS grade.

j. *Chromatographic Solvent.*—Skellysolve C:methanol:ethyl oleate, 94:4:2.

Procedure

Sample.—Pipet 1 ml steam-volatile nutmeg or mace oil (1, 5) into a 12 ml centrifuge tube, and add 5 ml $CHCl_3$ and 4 ml 10% NaOH. Shake well and centrifuge briefly to separate layers. Pipet 2 ml of the basic layer into a wide test tube and cool carefully in ice-water bath. Keep cold.

Preparation of Diazonium Salt.—Dissolve 0.276 g p-nitroaniline in 5 ml 24*N* H_2SO_4 in a 250 ml beaker. Add 5 ml H_2O and 3 g solid NaOH. Cool and add 20 g ice. Place the beaker in ice-salt bath, and with continuous stirring, add slowly (over 15 min.) 10 ml 0.2*M* $NaNO_2$ from a buret. Stir for 15 min. after adding nitrite, and add 20 g ice and 40 ml ice-cold H_2O. Keep cold.

Coupling of Phenols.—With a cold pipet add 4 ml of the diazonium salt solution to the basic solution of phenols, slowly with shaking. Keep the test tube cold during the addition of diazonium salt.

Measurement of Absorbance.—Dilute 1 ml of the solution of coupled phenols to 10 ml with H_2O. Measure the absorbance at

[1] Mention of firms or trade names in this paper does not constitute endorsement by the U. S. Department of Agriculture over similar products not mentioned.

398 mμ against a blank derived from 2 ml 10% NaOH carried through the coupling step.

Chromatography

Dilute the remainder of the coupled phenol solution to about 10 ml with H_2O. Add 5 ml $CHCl_3$ and shake. Remove most of the aqueous layer with a medicine dropper, acidify rapidly with 6*N* H_2SO_4, and add 1–2 ml $CHCl_3$. Shake, let layers separate, and pipet out the $CHCl_3$ layer. Dry over Na_2SO_4 and concentrate to small volume (warm water or nitrogen stream).

Place a small spot (0.5 cm diameter) of the $CHCl_3$ solution about 5 cm from one end of a strip of Whatman No. 1 filter paper, 1 ×13 cm. Insert this end of the strip into a wide test tube (7) containing about 10 ml of the chromatographic solvent mixture, supporting the upper end of the strip on a wire hook attached to a cork. Stopper the test tube with this cork, being careful not to let the strip touch the sides of the test tube. Initially the spot should be about 2 cm above the surface of the solvent.

Let the chromatogram run for about 10 min., until the solvent has almost reached the upper end of the strip. Remove the strip, mark the position of the solvent front, dry briefly in air, and spray with NaOH solution. Note R_F values and colors of spots.

Results and Discussion

The spectrophotometric measurement of the dyes formed from phenolic compounds present in nutmeg or mace oils serves to distinguish the origins of the spice samples.

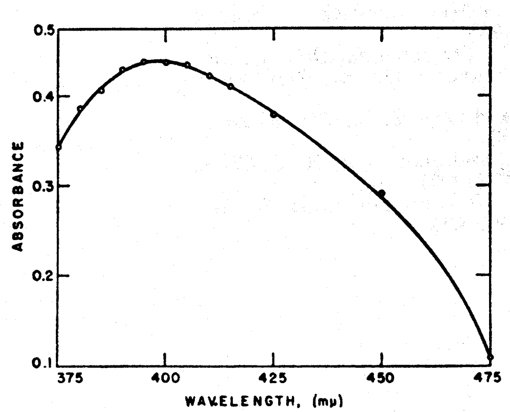

Fig. 1. Absorption spectrum of azo dye mixtures derived from phenols present in nutmeg and mace oils.

Table 1. Spectrophotometric identification of nutmeg or mace sources

Sample	Absorbance at 398 mμ
West Indian Nutmeg, I	0.000
West Indian Nutmeg, II	0.004
East Indian Nutmeg, I	0.132
East Indian Nutmeg, II	0.151
East Indian Nutmeg, III	0.148
West Indian Mace	0.020
East Indian Mace, I	0.142
East Indian Mace, II	0.133

The East Indian oils contain much greater quantities of these phenols than do the West Indian oils.

The spectra of the dye mixtures show a broad peak with a maximum at 398 mμ (Fig. 1). The spectrum in Fig. 1 was obtained from an East Indian nutmeg oil, the final dilution being less than in the analytical procedure described above.

Table 1 summarizes results obtained with a number of authentic samples of nutmeg and mace. All of these samples were from whole spices ground in the laboratory and oil prepared by steam distillation (5). From the data in Table 1 it can be seen that the origin of a nutmeg or mace sample can be readily ascertained from the single absorbance measurement. If the absorbance is not much different from the blank, then the sample is of West Indian origin; if the absorbance is much higher than the blank, then the sample is of East Indian origin.

Paper chromatography of the coupled phenols provides a second method for distinguishing the origin of a spice sample. It is not as rapid as the spectrophotometric procedure, because extra time is needed to isolate the dyes, but it has the advantage of requiring only simple, readily available apparatus.

The chromatographic system was designed primarily to detect the origin of a sample, rather than to completely separate the component dyes. In other solvent systems (e.g., amyl alcohol-aqueous methyl amine) better separation of individual compounds is obtained, but with an accompanying increase in time needed for good resolution. In the rapid chromatographic procedure described above, samples of East Indian

Table 2. Chromatographic identification of nutmeg or mace source

Sample	R_F Value	Color
West Indian Nutmeg and West Indian Mace	0.1	Pink
	0.9	Yellow-orange
East Indian Nutmeg and East Indian Mace	0.0	Green
	0.9	Purple

Table 3. Relative absorbance of p-nitrobenzeneazoeugenol prepared at various pHs

pH of Reaction Mixture	Relative Absorbance at 398 mμ
Below 5.0	0[a]
6.0	0.10[a]
7.0	0.47
8.2	1.00
9.2	0.75
10.0	0.48
Above 10.0	0.43

[a] Solution made basic with sodium hydroxide prior to spectrophotometric measurement for full color development.

origin are easily distinguished from those of West Indian origin by differences in color and position of the zones with low R_F values, as shown in Table 2.

The zones listed in Table 2 are not the only ones obtainable, but they are sufficient to determine the source of a sample. Actually, at least six phenolic compounds have been detected by chromatography of East Indian oils in the amyl alcohol-methyl amine system and four or five from West Indian oils. In both types of oil, eugenol is the predominant phenol present. One of the other phenols found is isoeugenol, although there is a possibility that the iso compound is an artifact produced by isomerization of eugenol in basic solution (4). Other phenols present have not yet been identified, but probably include phenols related to myristicin and safrole, which have been shown to be present in nutmeg volatile oil (6).

Similarly, the spectrophotometric method is empirical in the sense that not all the phenolic compounds are quantitatively coupled with diazonium salt. The amount of eugenol present is much greater than the sum of all other phenolic compounds present, and if the reaction conditions were such as to allow quantitative reaction of eugenol, then the absorbance due to eugenol alone would obscure the smaller differences due to source variation.

The data in Table 3 show that the extent of reaction of eugenol with the diazonium salt varies with *p*H, decreasing from a maximum at *p*H 8.2, not only as the reaction mixture is made more acidic, but also as it is made more basic. Fortunately, other phenolic compounds present in the oils do not exhibit this behavior, at least not to the same extent, so that by carrying out the coupling step at a very high *p*H, the differences between East and West Indian sources are preserved, while the obscuring effect of the large amounts of eugenol is decreased.

Acknowledgment

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